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(54) METHOD FOR THE PRODUCTION OF FREEZE-DRIED PELLETS COMPRISING FACTOR VIII

(57) A method for the production of freeze-dried pellets comprising factor VIII comprises the steps of:
a) freezing droplets of a solution comprising factor VIII to form pellets;
b) freeze-drying the pellets;

wherein in step a) the droplets are formed by means of droplet formation of the solution comprising factor VIII into a cooling tower which has a temperature-controllable inner wall surface and an interior temperature below the freezing temperature of the solution and wherein in step b) the pellets are freeze-dried in a rotating receptacle which is housed inside a vacuum chamber.

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Description

[0001] The present invention relates to a method for the production of freeze-dried pellets comprising factor VIII, the method comprising the steps of: a) freezing droplets of a solution comprising factor VIII to form pellets; and b) freeze-drying the pellets.

[0002] Factor VIII (FVIII) is a protein found in blood plasma, which acts as a cofactor in the cascade of reactions leading to blood coagulation. A deficiency in the amount of FVIII activity in the blood results in the clotting disorder known as hemophilia A, an inherited condition primarily affecting males. Hemophilia A is currently treated with therapeutic preparations of FVIII derived from human plasma or manufactured using recombinant DNA technology. Such preparations are administered either in response to a bleeding episode (on-demand therapy) or at frequent, regular intervals to prevent uncontrolled bleeding (prophylaxis).

[0003] A conventional process for manufacturing and packaging parenteral biopharmaceuticals involves the formulation of a bulk solution in accordance with the measured biological activity of the intermediate material used to formulate the bulk solution. In many cases, particularly at the end of the process, the bulk solution is frozen and stored for making the assay. For this purpose the frozen solution may be stored for several days or even for several weeks. For the subsequent filling of the final packages, such as vials, for distribution to the end users, the frozen intermediate solution is typically thawed, bulked and filled into vials, and then freeze-dried within the vials.

[0004] The amount of thawed bulk solution that is filled into the final packaging vials is calculated on the basis of the assay of the intermediate solution. This calculation usually incorporates a large safety margin because of (1) large variation of biological assay and (2) loss of yield in the subsequent sterile fill and freeze-drying process. The loss of yield is due to product stress during this first freezing, storing and thawing step and the following second filling, freezing and drying process. This calculation is of course very difficult and based on product dependent empirical knowledge of the complete process.

[0005] In conventional processes the freeze-drying is usually performed in standard freeze drying chambers which do not have temperature controlled walls. These dryers, unfortunately, provide non-homogeneous heat transfer to the vials placed in the dryer chamber. Especially those vials which are positioned at the edges exchange energy more intensively than those positioned in the center of the plates, due to radiant heat exchange and natural convection in the gap between the wall of the chamber and the stack of plates/shelves. This non-uniformity of energy distribution leads to a variation of freezing and drying kinetics between the vials at the edges and those in the center, and could result in variation in the activities of the active contents of the respective vials. To ensure the uniformity of the final product, it is necessary to conduct extensive development and validation work both at laboratory and production scales.

[0006] The publication by Wang, D. Q., MacLean, D. and Ma, X. (2010) entitled Process Robustness in Freeze Drying of Biopharmaceuticals, in Formulation and Process Development Strategies for Manufacturing Biopharmaceuticals (eds F. Jameel and S. Hershenson), John Wiley & Sons, Inc., Hoboken, NJ, USA discloses specific freeze-drying cycles for recombinant FVIII but still acknowledges potency variations as a function of the vial position in the freeze-drying chamber.

[0007] WO 2010/054238 A1 reports on a stable lyophilized pharmaceutical formulation of Factor VIII (FVIII) comprising: (a) a FVIII; (b) one or more buffering agents; (c) one or more antioxidants; (d) one or more stabilizing agents; and (e) one or more surfactants; said FVIII comprising a polypeptide selected from the group consisting of: a) a recombinant FVIII polypeptide; b) a biologically active analog, fragment or variant of a); said buffer is comprising of a pH buffering agent in a range of about 0.1 mM to about 500 mM and said pH is in a range of about 2.0 to about 12.0; said antioxidant is at a concentration of about 0.005 to about 1.0 mg/ml; said stabilizing agent is at a concentration of about 0.005 to about 20%; said surfactant is at a concentration of about 0.001% to about 1.0%; and said formulation excluding sodium chloride (NaCl) or including only trace amount of NaCl.

[0008] WO 2006/008006 A1 is concerned with a process for sterile manufacturing, including freeze-drying, storing, assaying and filling of pelletized biopharmaceutical products in final containers such as vials. A process for producing containers of a freeze-dried product is disclosed, the process comprising the steps of freezing droplets of the product to form pellets, freeze-drying the pellets, assaying the freeze-dried pellets and loading the freeze-dried pellets into containers. More specifically, the process comprises the steps of: a) freezing droplets of the product to form pellets, whereby the droplets are formed by passing a solution of the product through frequency assisted nozzles and pellets are formed from said droplets by passing them through a counter-current flow of cryogenic gas; b) freeze-drying the pellets; c) storing and homogenizing the freeze-dried pellets; d) assaying the freeze dried pellets while they are being stored and homogenized; and e) loading the freeze-dried pellets into said containers.

[0009] WO 2013/050156 A1 describes a process line for the production of freeze-dried particles under closed conditions comprising at least a spray chamber for droplet generation and freeze congealing of the liquid droplets to form particles and a bulk freeze-dryer for freeze drying the particles, the freeze-dryer comprising a rotary drum for receiving the particles. Further, a transfer section is provided for a product transfer from the spray chamber to the freeze-dryer. For the production of the particles under end-to-end closed conditions each of the devices and of the transfer section is separately adapted for operation preserving sterility of the product to be freeze-dried and/or containment.

[0010] WO 2013/050161 A1 discloses a process line for the production of freeze-dried particles under closed conditions, the process line comprising a freeze-dryer for the bulk ware production of freeze-dried particles under closed conditions, the freeze-dryer comprising a rotary drum for receiving the frozen particles, and a stationary vacuum chamber housing the rotary drum, wherein for the production of the particles under closed conditions the vacuum chamber is adapted for closed operation during processing of the particles. The drum is in open communication with the vacuum chamber and at least one transfer section is provided for a product transfer between a separate device of the process line and the freeze-dryer, the freeze-dryer and the transfer section being separately adapted for closed operation, wherein the transfer section comprises a temperature-controllable inner wall surface.

[0011] It would be desirable to produce freeze-dried pellets of factor VIII with fewer variations in activity for the individual pellets under conditions of strict separation from the outside - this includes any cryogenic gas such as liquid nitrogen. The present invention has the object of providing such a method.

[0012] This object is achieved according to the present invention by a method for the production of freeze-dried pellets comprising factor VIII, the method comprising the steps of:

a) freezing droplets of a solution comprising factor VIII to form pellets;

b) freeze-drying the pellets;

wherein in step a) the droplets are formed by means of droplet formation of the solution comprising factor VIII into a cooling tower which has a temperature-controllable inner wall surface and an interior temperature below the freezing temperature of the solution and in step b) the pellets are freeze-dried in a rotating receptacle which is housed inside a vacuum chamber.

[0013] The operating principle of the method according to the invention has two distinct advantages. Firstly, it should be noted that in the method according to the invention the sprayed droplets of the factor VIII-comprising solution do not contact a cryogenic gas such in a counter-flow fashion such as described in WO 2006/008006 A1. There is no need for introducing a cryogenic gas into the cooling tower and hence all handling and sterilization steps for the cryogenic gas can be omitted.

[0014] Secondly, by conducting the freeze-drying step in a rotating receptacle inside the vacuum chamber the spatial position of each individual pellet is evenly distributed over time. This ensures uniform drying conditions and therefore eliminates spatial variations of the activity of factor VIII as would be the case for freeze-dried vials on a rack.

[0015] It has been experimentally found that actual potencies of the pellets after freeze-drying are between 86.2% and 89.9% of the target potencies for factor VIII.

[0016] All steps of the method according to the invention can be carried out under sterile conditions and without compromising sterility between the individual steps.

[0017] Creation of frozen pellets can be performed with any of the known technologies, such as with a "Kryogen Rapid Pelletizer" from Messer-Griesheim, Germany or "CRYOGENIC PELLETIZER" from IQFCRYOGRAN, Canada. Due to the subsequent freeze drying step, the frozen pellets are expected to have a narrow particle size. Afterwards the frozen pellets can be transported under sterile and cold conditions to a freeze dryer. The pellets are then distributed across the carrying surfaces inside the drying chamber by the rotation of the receptacle. Sublimation drying is in principle possible in any kind of freeze dryers suited for pellets. Freeze dryers providing space for sublimation vapor flow, controlled wall temperatures and suitable cross sectional areas between drying chamber and condenser are preferred.

[0018] Details of the factor VIII variants which can be employed in the method according to the invention are described below. Preferably, a recombinant factor VIII derived from baby hamster kidney cells without additional proteins present is used.

[0019] Herein, the term "Factor VIII" or "FVIII" or "rAHF" refers to any FVIII molecule which has at least a portion of the B domain intact, and which exhibits biological activity that is associated with native FVIII. In one embodiment of the disclosure, the FVIII molecule is full-length FVIII. The FVIII molecule is a protein which is encoded for by DNA sequences capable of hybridizing to DNA encoding FVIILC. Such a protein may contain amino acid deletions at various sites between or within the domains A1-A2-B-A3-C1-C2 (U.S. Pat. No. 4,868,112). The FVIII molecule may also be an analog of native FVIII wherein one or more amino acid residues have been replaced by site-directed mutagenesis.

[0020] According to the present disclosure, the term "recombinant Factor VIII " (rFVIII) may include any rFVIII, heterologous or naturally occurring, obtained via recombinant DNA technology, or a biologically active derivative thereof. In certain embodiments, the term encompasses proteins as described above and nucleic acids, encoding a rFVIII of the disclosure. Such nucleic acids include, for example and without limitation, genes, pre-mRNAs, mRNAs, polymorphic variants, alleles, synthetic and naturally-occurring mutants. Proteins embraced by the term rFVIII include, for example and without limitation, those proteins and polypeptides described hereinabove, proteins encoded by a nucleic acid described above, interspecies homologs and other polypeptides that: (1) have an amino acid sequence that has greater than about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about

93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% or greater amino acid sequence identity, over a region of at least about 25, about 50, about 100, about 200, about 300, about 400, or more amino acids (up to the full length sequence of 2332 amino acids for the mature native protein), to a polypeptide encoded by a referenced nucleic acid or an amino acid sequence described herein; and/or (2) specifically bind to antibodies, e.g., polyclonal or monoclonal antibodies, generated against an immunogen comprising a referenced amino acid sequence as described herein, an immunogenic fragment thereof, and/or a conservatively modified variant thereof.

[0021] As used herein, "endogenous FVIII" includes FVIII which originates from the mammal intended to receive treatment. The term also includes FVIII transcribed from a transgene or any other foreign DNA present in said mammal. As used herein, "exogenous FVIII" includes FVIII which does not originate from said mammal.

[0022] The FVIII molecule exists naturally and in therapeutic preparations as a heterogeneous distribution of polypeptides arising from a single gene product (see, e.g., Andersson et al., Proc. Natl. Acad. Sci. USA, 83, 2979 2983, May 1986). The term "Factor VIII" as used herein refers to all such polypeptides, whether derived from blood plasma or produced through the use of recombinant DNA techniques and include, but is not limited to FVIII mimetics, fc-FVIII conjugates, FVIII chemically modified with water soluble polymers and other forms or derivatives of FVIII. Commercially available examples of therapeutic preparations containing FVIII include those sold under the trade names of HEMOFIL M and RECOMB INATE (available from Baxter Healthcare Corporation, Deerfield, Ill., U.S.A.). Other preparations comprise primarily a single subpopulation of FVIII molecules, which lack the B domain portion of the molecule.

[0023] The starting material of the present disclosure is FVIII, which can be derived from human plasma, or produced by recombinant engineering techniques, as described in patents U.S. Pat. No. 4,757,006; U.S. Pat. No. 5,733,873; U.S. Pat. No. 5,198,349; U.S. Pat. No. 5,250,421; U.S. Pat. No. 5,919,766; EP 306 968.

[0024] The FVIII molecules useful for the present disclosure include the full-length protein, precursors of the protein, biologically active or functional subunits or fragments of the protein, and functional derivatives thereof, as well as variants thereof as described herein below. Reference to FVIII is meant to include all potential forms of such proteins and wherein each of the forms of FVIII has at least a portion or all of the native B domain sequence intact.

[0025] Polynucleotides encoding a rFVIII of the disclosure include, without limitation, those that (1) specifically hybridize under stringent hybridization conditions to a nucleic acid encoding a referenced amino acid sequence as described herein, and conservatively modified variants thereof; (2) have a nucleic acid sequence that has greater than about 95%, about 96%, about 97%, about 98%, about 99%, or higher nucleotide sequence identity, over a region of at least about 25, about 50, about 100, about 150, about 200, about 250, about 500, about 1000, or more nucleotides (up to the full length sequence of 6996 nucleotides of the mature protein), to a reference nucleic acid sequence as described herein.

[0026] Variant (or analog) polypeptides include insertion variants, wherein one or more amino acid residues are added to an FVIII amino acid sequence of the disclosure. Insertions may be located at either or both termini of the protein, and/or may be positioned within internal regions of the FVIII amino acid sequence. Insertion variants, with additional residues at either or both termini, include for example, fusion proteins and proteins including amino acid tags or other amino acid labels. In one aspect, the FVIII molecule may optionally contain an N-terminal Met, especially when the molecule is expressed recombinantly in a bacterial cell such as E. coli.

[0027] In deletion variants, one or more amino acid residues in a FVIII polypeptide as described herein are removed. Deletions can be effected at one or both termini of the FVIII polypeptide, and/or with removal of one or more residues within the FVIII amino acid sequence. Deletion variants, therefore, include all fragments of a FVIII polypeptide sequence.

[0028] In substitution variants, one or more amino acid residues of a FVIII polypeptide are removed and replaced with alternative residues. In one aspect, the substitutions are conservative in nature and conservative substitutions of this type are well known in the art. Alternatively, the disclosure embraces substitutions that are also non-conservative. Exemplary conservative substitutions are described in Lehninger, [Biochemistry, 2nd Edition; Worth Publishers, Inc., New York (1975), pp.71-77] and set out immediately below.

[0029] Embodiments and additional aspects of the present invention will be described below. They can be combined freely unless the context clearly indicates otherwise.

[0030] For the present invention any deleted, substituted and/or other variation of the FVIII polypeptide may be processed without the need for further variation of the process itself. It's however relevant to the process that a FVIII polypeptide is processed, because it's specific to the resulting FVIII formulations that they comprise only a minor fraction of the actual FVIII polypeptide in comparison to the overall amount of constituents therein.

[0031] From that it's apparent that the process must be such that any potential damage to the FVIII polypeptide freeze-dried as a part of the formulation is avoided because a slight decrease of the FVIII polypeptide activity in the finally freeze-dried formulation results in an percentage wise huge impact of activity in the final product.

[0032] In one embodiment of the method according to the invention the method further comprises the steps c), d) and e) after step b):

c) storing and homogenizing the freeze-dried pellets

d) assaying the freeze dried pellets while they are being stored and homogenized;

e) loading the freeze-dried pellets into containers.

[0033] The storing and homogenization step c) can also be performed in the rotating receptacle within the vacuum chamber used for freeze-drying. A statistically relevant number of samples are extracted for performing an assay. After that the optional separate storage container is packed into a sterile containment. After assaying the content of each storage container all necessary properties such as e.g. actives content are known. The filling process into the final containers with the user defined amounts of pellets can then begin. The storage containers are transferred to an isolated filling line and docked at a sterile docking station. The contents of the containers are transferred inside the isolator to the storage of the filling machine.

[0034] In another embodiment of the method according to the invention in step a) the droplets are formed by means of droplet formation of the solution by passing through frequency-assisted nozzles. Preferably the oscillating frequency is ≥ 1000 Hz to ≤ 2000 Hz.

[0035] In another embodiment of the method according to the invention in step a) the inner surface of the cooling tower has a temperature of not more than -120°C, preferably ≥ -150 °C to ≤ -120 °C. Preferably the temperature is ≥ -140 °C to ≤ -130 °C.

[0036] The above referred to temperatures of ≥ -140 °C to ≤ -130 °C are optimized for droplet sizes in the range of ≥ 700 μm to ≤ 900 μm that are frozen while falling a distance of 3m to 4m.

[0037] As long as the temperature is kept below -120°C on the inner surface of the cooling tower, the beneficial results of the present invention can be obtained by adjustment of falling distance and droplet size.

[0038] In another embodiment of the method according to the invention the inner surface of the cooling tower is cooled by passing a coolant through one or more pipes which are in thermal contact with the inner surface. The coolant may be liquid nitrogen or nitrogen vapor of a desired temperature.

[0039] In another embodiment of the method according to the invention a target dosage is established for factor VIII, the assay in step d) determines the active content of factor VIII in the freeze-dried pellets and the containers are loaded with an amount of freeze-dried pellets that provides a dosage which equals the target dosage, or exceeds the target dosage by ≤ 25%. Preferably the target dosage is exceeded by ≤ 10%, more preferably by ≤ 5%.

[0040] It is a direct result of the present invention allowing a gentle freezing of the FVIII polypeptide that allows not to dramatically exceed the target dosage upon filling, because the process does not decrease the activity of the FVIII polypeptide.

[0041] In another embodiment of the method according to the invention the pellets obtained in step a) have a maximum of the particle size distribution d50 of ≥ 200 μm to ≤ 1500 μm. Preferred is a maximum of the particle size distribution d50 of ≥ 700 μm to ≤ 900 μm.

[0042] Pellets of smaller size than 200 μm are less favorable as in those pellets freezing would be faster which may result in damages of the freeze dried polypeptide and thus loss in potency requiring higher target dosage. Furthermore electrostatic influences of the resulting powder increase dramatically at sizes below 200 μm leading to inferior handling properties of the product of the present process.

[0043] Increase of pellet size to more than 1500 μm may endanger complete freezing of the pellet in the described setup and thus impair the overall efficacy of a later product.

[0044] In another embodiment of the method according to the invention the solution comprising factor VIII in step a) has a content of dissolved solids of ≥ 8 weight-% to ≤ 12 weight-%. Preferred is a content of dissolved solids of ≥ 9 weight-% to ≤ 11 weight-%.

[0045] In principle higher loads of dissolved solids of about 15 to 20 weight-% would be deemed favorable in processes of the kind concerned herein because the resulting pellets would normally be expected to be frozen more easily and dried more robustly.

[0046] However, in the present case the above ranges are found to be better as the handled solid (comprising FVIII) later needs to be reconstituted and injected into a human being which (at higher loads) would either result in an incompatible osmolality of the injection solution resulting in potential tissue damage and/or injection pain or otherwise would require significantly higher reconstitution volumes to avoid that which in fact renders the solution no longer practically injectable.

[0047] In another embodiment of the method according to the invention the solution comprising factor VIII in step a) has the following composition with respect to 1 gram of the solution, the balance being water for injection:

Factor VIII	≥ 99 IU to ≤ 101 IU,
Sucrose	≥ 68 mg to ≤ 72 mg, preferably ≥ 70 mg to ≤ 71.8 mg
Histidine	≥ 2 mg to ≤ 4 mg, preferably ≥ 3 mg to ≤ 3.8 mg

(continued)

Glycine	≥ 23 mg to ≤ 26 mg, preferably ≥ 23.5 mg to ≤ 25.7 mg
NaCl	≥ 1 mg to ≤ 3 mg, preferably ≥ 1.5 mg to ≤ 2.5 mg
$CaCl_2$	≥ 0.2 mg to ≤ 0.4 mg, preferably ≥ 0.25 mg to ≤ 0.35 mg
Polysorbate 80	≥ 0.07 mg to ≤ 0.1 mg, preferably ≥ 0.075 mg to ≤ 0.095 mg

[0048] The present invention will be further described with reference to the following figures and examples without wishing to be limited by them.

FIG. 1 schematically shows an apparatus for the method according to the invention

FIG. 2 shows the temperature profile over time in a cooling tower

FIG. 3 shows a temperature and pressure profile during a freeze-drying step

FIG. 4 shows another temperature and pressure profile during a freeze-drying step

[0049] FIG. 1 schematically depicts an apparatus for conducting the method according to the invention. The apparatus comprises, as main components, the cooling tower 100 and the vacuum drying chamber 200. The cooling tower comprises an inner wall 110 and an outer wall 120, thereby defining a space 130 between the inner wall 110 and the outer wall 120.
[0050] This space 130 houses a cooling means 140 in the form of piping. A coolant can enter and leave the cooling means 140 as indicated by the arrows of the drawing.
[0051] Coolant flowing through the cooling means 140 leads to a cooling of the inner wall 110 and thus to a cooling of the interior of the cooling tower 100. In the production of frozen pellets (cryopellets), liquid is sprayed into the cooling tower via nozzle 150. Liquid droplets are symbolized in accordance with reference numeral 160.
[0052] The liquid droplets eventually solidify (freeze) on their downward path, which is symbolized in accordance with reference numeral 170. Frozen pellets 170 travel down a chute 180 where a valve 190 permits entry into the vacuum drying chamber 200.
[0053] While not depicted here, it is of course also possible and even preferred that the chute 180 is temperature-controlled in such a way as to keep the pellets 170 in a frozen state while they are collecting before the closed valve 190.
[0054] Inside the vacuum drying chamber 200 a rotatable drum 210 is located to accommodate the frozen pellets to be dried. The rotation occurs around the horizontal axis in order to achieve an efficient energy transfer into the pellets. As an end result, freeze-dried pellets symbolized by the reference numeral 220 are obtained.

Example 1

[0055] Cryopellets of a solution of Kogenate® PF were manufactured. Kogenate® PF is a plasma protein-free recombinant human factor VIII. The formulation for 1 g of the solution is given below:

Solids	**Target: 10%**	**Actual: 10,3 %**
Kogenate® PF	100 IU	100 IU
Sucrose	70,87 mg	71,79 mg
Histidine	3,32 mg	3,59 mg
Glycine	23,6 mg	25,54 mg
Sodium chloride	1,88 mg	2,03 mg
Calcium chloride	0,28 mg	0,30 mg
Polysorbate 80	0,08 mg	0,09 mg
Water for injection	ad 1 g	ad 1g

[0056] The bulk solution was sprayed into a wall-cooled cooling tower in accordance with the method according to the invention. The spraying nozzle had one aperture with a diameter of 400 μm. This corresponds to target droplet size of 800 μm. The oscillation frequency was 1375 Hz, the deflection pressure 0.2 bar and the pump was operated at 22 rpm.

After a total duration of 35 minutes 879.3 g of frozen pellets were collected (96% yield).

[0057] The interior temperatures of the cooling tower were monitored and their development over time is depicted in FIG. 2. Curve 1000 represents the sensor reading from an upper part of the cooling tower, curve 1010 the sensor reading from a central part of the cooling tower and curve 1020 the sensor reading from a lower part of the cooling tower. At 14:45 o'clock, when the temperatures had reached -126.0 °C (upper sensor), -129.7 °C (central sensor) and -133.1 °C (lower sensor), the spraying operation was initiated. This is represented by mark 1030 in FIG. 2. At 15:20 o'clock the spraying operation was halted (mark 1040) with recorded temperatures of -130.7 °C (upper sensor), -133.5 °C (central sensor) and -135.2 °C (lower sensor). The frozen pellets were collected in a cooled container having a temperature between -55 °C and -53 °C.

Example 2

[0058] This example concerns the freeze-drying of a sample of cryopellets obtained in example 1. A LyoMotion freeze-dryer from Meridion was employed in this step. This machine comprises a rotating drum in which the cryopellets were agitated and subjected to drying.

[0059] A total of 21.3 g freeze-dried pellets (72.6% yield) having a residual moisture of 0.95% were isolated.

[0060] The temperature and pressure profiles of the freeze-drying step are shown in FIG. 3. Curve 1050 represents the product temperature, curve 1060 the condenser temperature of the freeze-drying machine and curve 1070 the internal pressure inside the vacuum chamber of the freeze-drying machine.

Example 3

[0061] This example concerns the freeze-drying of another sample of cryopellets obtained in example 1. A LyoMotion freeze-dryer from Meridion was employed in this step. This machine comprises a rotating drum in which the cryopellets were agitated and subjected to drying.

[0062] A total of 21.4 g freeze-dried pellets (73.7% yield) having a residual moisture of 0.70% were isolated.

[0063] The temperature and pressure profiles of the freeze-drying step are shown in FIG. 4. Curve 1080 represents the product temperature, curve 1090 the condenser temperature of the freeze-drying machine and curve 1100 the internal pressure inside the vacuum chamber of the freeze-drying machine.

Results

[0064] Potency assays and size exclusion chromatography analyses of the products obtained are given in the table below.

		Size exclusion chromatography (average of 2 samples) [relative area-%]		
Sample	Potency [% of target potency]	Region 1 (HMW)	Region 2	Region 3
Ex. 2	86,2 %	0,6	75,0	17,2
Ex. 2	89,9 %	0,5	74,6	17,5
Ex. 3	87,9 %	0,5	73,8	18,3
Ex. 3	88,4 %	0,5	74,5	17,6

[0065] Two samples from each example were analyzed with respect to the potency of the factor VIII therein. Target potencies were 250.0 mg/vial. A loss of potency during the processing of the bulk solution and freeze-drying is to be expected. The determined actual potencies between 86.2% and 89.9% were considerably lower in variation than those observed in conventional freeze-drying in a vial. Here potencies ranging from 80.9% to 91.2% can be observed depending on the position of the individual vial in the drying chamber.

[0066] For reference, the following table gives analytical data for the precursors of examples 2 and three (IPC values).

			Size exclusion chromatography (average of 2 samples) [relative area-%]		
Sample	Target potency [IU/ml]	Potency [% of target potency]	Region 1 (HMW)	Region 2	Region 3
Kogenate® PF solution after thawing	1220.0	101,2%	0,4	74,5	17,6
Kogenate® PF bulk solution after dilution	100.0	96,8 %	0,6	73,6	17,5
Ex. 1 (cryopellets)	100.0	111,7 %	0,6	73,0	18,0

Claims

1. A method for the production of freeze-dried pellets comprising factor VIII, the method comprising the steps of:

a) freezing droplets of a solution comprising factor VIII to form pellets;
b) freeze-drying the pellets;

characterized in that
in step a) the droplets are formed by means of droplet formation of the solution comprising factor VIII into a cooling tower (100) which has a temperature-controllable inner wall surface (110) and an interior temperature below the freezing temperature of the solution
and that
in step b) the pellets are freeze-dried in a rotating receptacle (210) which is housed inside a vacuum chamber (200).

2. The method according to claim 1, further comprising the steps c), d) and e) after step b):

c) storing and homogenizing the freeze-dried pellets
d) assaying the freeze dried pellets while they are being stored and homogenized;
e) loading the freeze-dried pellets into containers.

3. The method according to claim 1 or 2, wherein in step a) the droplets are made by means of droplet formation by passing the solution through frequency-assisted nozzles.

4. The method according to claim 3, wherein the oscillating frequency is ≥ 1000 Hz to ≤ 2000 Hz.

5. The method according to one of claims 1 to 4, wherein in step a) the inner surface (110) of the cooling tower (100) has a temperature of ≤ -120 °C.

6. The method according to one of claims 1 to 5, wherein the inner surface (110) of the cooling tower (100) is cooled by passing a coolant through one or more pipes (140) which are in thermal contact with the inner surface (110).

7. The method according to one of claims 2 to 6, wherein a target dosage is established for factor VIII, the assay in step d) determines the active content of factor VIII in the freeze-dried pellets and the containers are loaded with an amount of freeze-dried pellets that provides a dosage which equals the target dosage, or exceeds the target dosage by ≤ 25%.

8. The method according to one of claims 1 to 7, wherein the pellets obtained in step a) have a maximum of the particle size distribution d50 of ≥ 200 μm to ≤ 1500 μm.

9. The method according to one of claims 1 to 8, wherein the solution comprising factor VIII in step a) has a content of dissolved solids of ≥ 8 weight-% to ≤ 12 weight-%.

10. The method according to one of claims 1 to 9, wherein the solution comprising factor VIII in step a) has the following composition with respect to 1 gram of the solution, the balance being water for injection:

Factor VIII	≥ 99 IU to ≤ 101 IU
Sucrose	≥ 68 mg to ≤ 72 mg
Histidine	≥ 2 mg to ≤ 4 mg
Glycine	≥ 23 mg to ≤ 26 mg
NaCl	≥ 1 mg to ≤ 3 mg
$CaCl_2$	≥ 0.2 mg to ≤ 0.4 mg
Polysorbate 80	≥ 0.07 mg to ≤ 0.1 mg

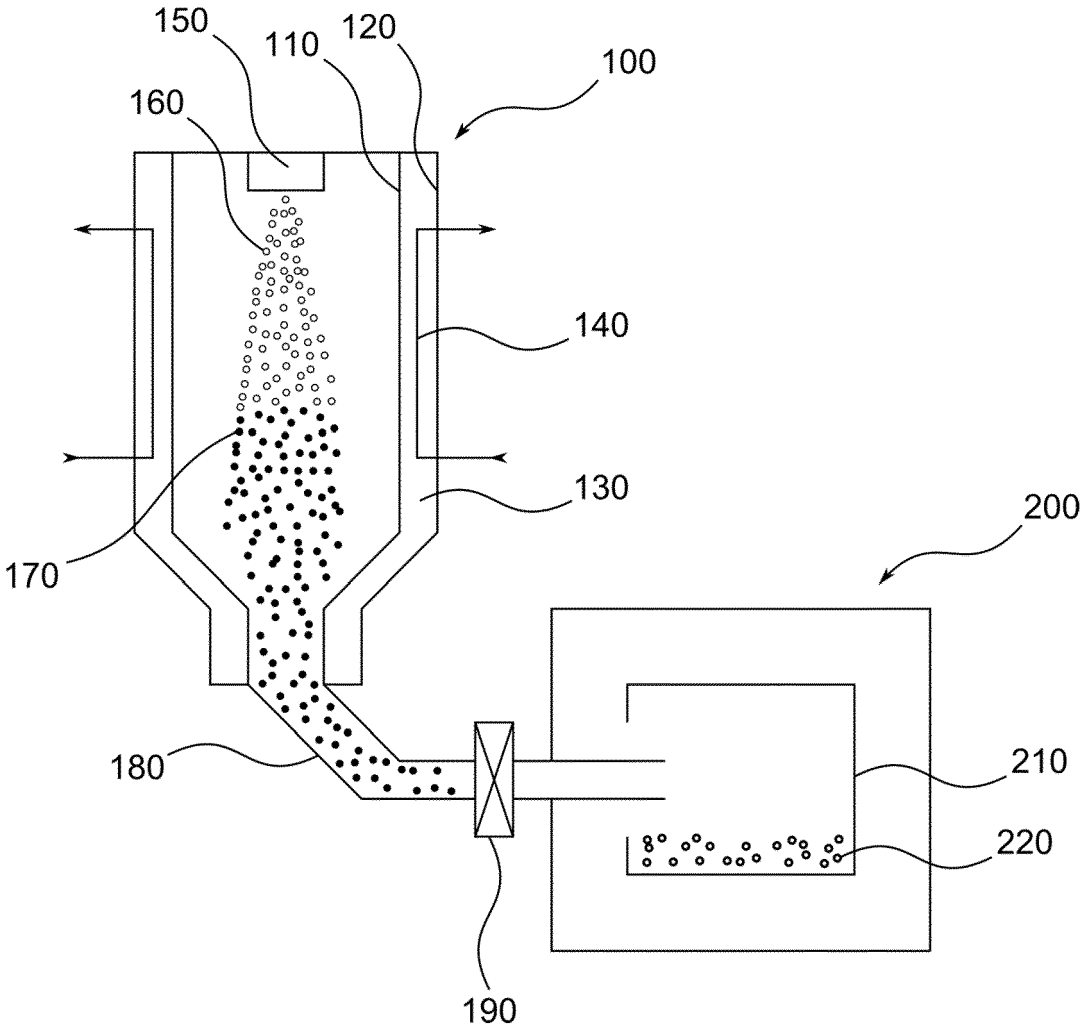
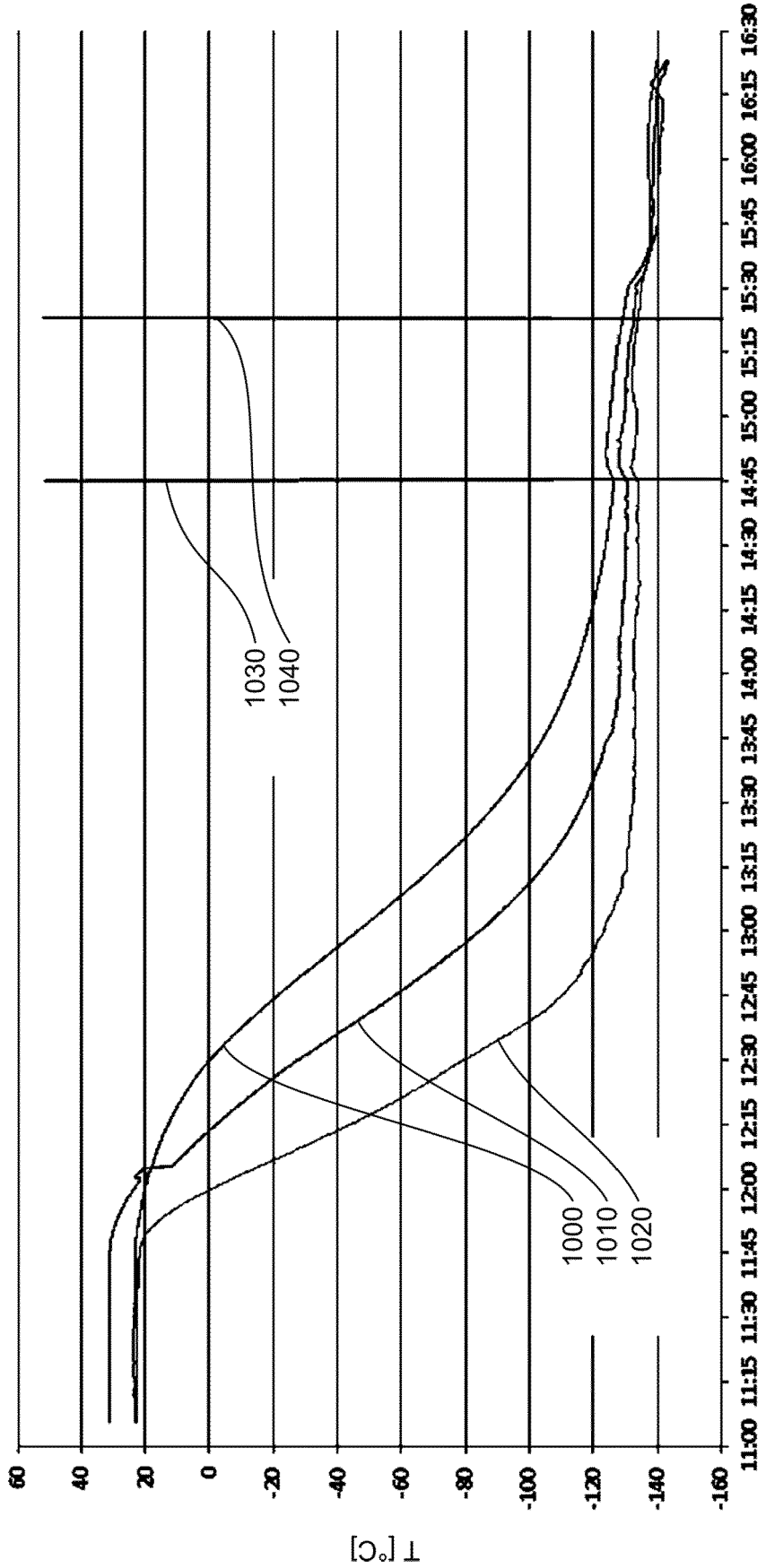


FIG. 1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-160
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1010
1020

FIG. 2

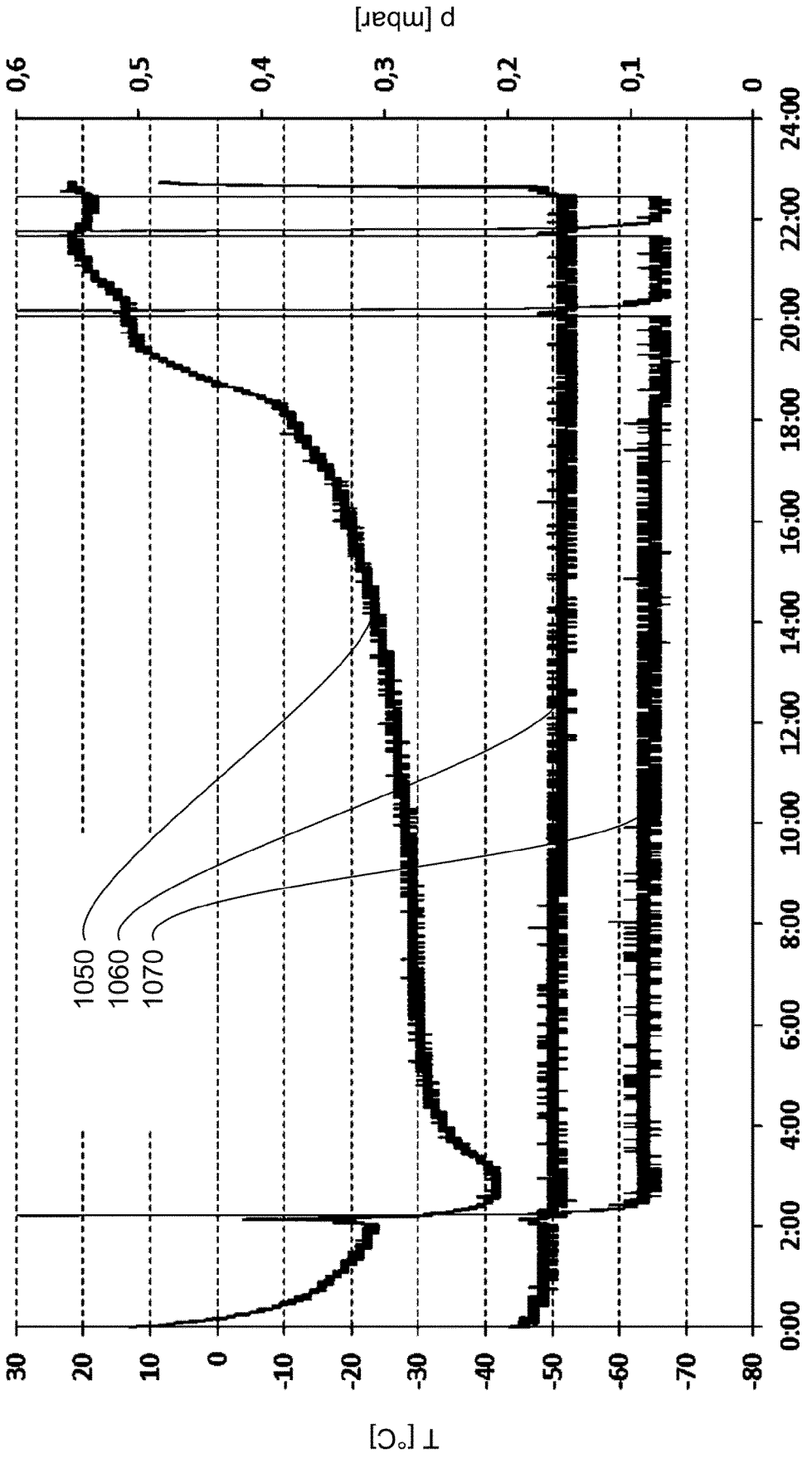
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FIG. 3

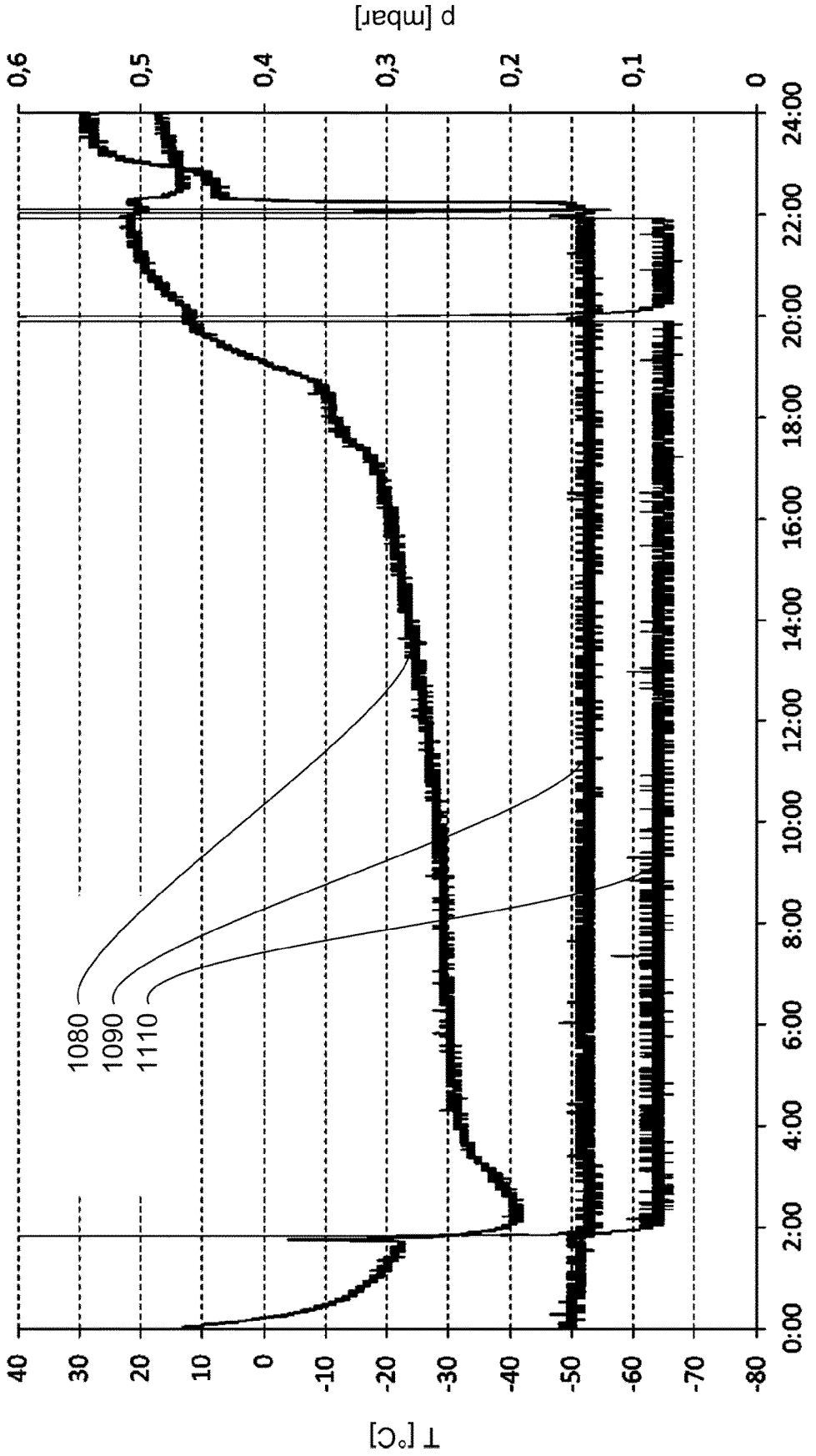
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FIG. 4

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EUROPEAN SEARCH REPORT

Application Number
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A61K
C07K
F26B
A61M

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	18 February 2016	Kardas-Llorens, Eyüp

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